

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Appl. No. : 10/600,266

Confirm. No. : 7488

Applicant(s) : Fumitoshi ASAI et al

Filed : June 20, 2003

Art Unit : 1614

Examiner : Brian Yong S. Kown

Docket No. : 03337C/HG

For : MEDICINAL COMPOSITIONS CONTAINING ASPIRIN

Customer No. : 01933

DECLARATION UNDER 37 CFR 1.131

I, Dr. Fumitoshi ASAI, hereby declare the following:

1. I am a co-inventor of the invention described and claimed in the above-identified application.
2. Attached hereto are copies of notebook records documenting experiments done by us (the inventors) or under our supervision and control, showing a reduction to practice of the claimed invention. The code "CS 747" which appears throughout the notebook pages is our internal code for the compound identified as "Compound A" in Table I of the specification of our patent application. The dates on the copies have been blacked out. Translations of these documents are attached. The acts described in these documents occurred prior to November 3, 1998.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Oct 11, 2007



Dr. Fumitoshi Asai

Attachment: Notebook records and
English translation thereof

145

CS 747
+ Aspirin) 併用 Exp. 行なう。

Dose CS 747 0.3 mg/kg (4hr) 2- 約 40-7.
1 mg/kg (4hr) 2- 約 20

出血時間、凝集とも行なう。 doe. 12

Aspirin 10 + CS 747 0.6 2hr

まず Aspirin 10 + CS 747 0.6 を中心として行なう。

± 1.2 ② ~ ① ① は多少強すぎたって ④ を行なう。

147

ラット

ロンアルファA、三共)で接着する。また12 cmのチューブ内に10 cmの綱糸(3-0、日腸工業)を設置する。

(3) 動静脈シャント開始2時間前に、vehicle (5%アラビアゴム溶液) または薬物を1 ml/kgの割合で経口投与する。1群6匹の実験を行う。

(4) あらかじめ作成しておいた上記チューブに30 unit/kgとなるように、生理食塩液(大塚)で希釈したヘパリン溶液(日本薬局方ヘパリンナトリウム注射液、扶桑薬品工業、Lot No. 97H28A、[REDACTED]入荷)を溝たす。

(5) 生理食塩液で40 mg/mlに希釈したペントバルビタール溶液(エンブタール®、Abbott、Lot No. 20-975-Z7)を1 ml/kg腹腔内投与(40 mg/kg)してラットを麻酔させる。仰臥位に固定させた後、頸静脈を露出させ、シャント用チューブの片側(糸の付いていない方)をカニュレーションする。続いて、クレンメで血流を遮断しておいた頭動脈に、チューブの反対端をカニュレーションし、動静脈シャントを形成する。

(6) クレンメをはずしてシャントに30分間血液を循環させた後、綱糸に付着した血栓の湿重量を測定する。測定重量から糸の重量(6.5 mg)を差し引き血栓重量を求める。

ファイル AV-shunt(3) 1に保存
(F00515テ-タ)

[REDACTED] A: Sugita

149

B.W. (g)	Treatment	測定値 (ms)	Thrombus (mg)
#1 253	Vehicle	61.3	54.8
2 252	Aspirin (10)	51.3	44.8
3 262	CS747 (0.7)	58.3	51.8
4 267	CS747 (0.6)	43.1	36.6
5 256	Aspirin (10) + CS747 (0.3)	39.6	33.1
6 271	Aspirin (10) + CS747 (0.9)	23.2	16.7
7 250	V	63.2	56.7
8 246	A (10)	58.4	51.9
9 258	747 (0.2)	51.8	45.3
10 269	747 (0.6)	53.1	46.6
11 268	A (10) + 747 (0.3)	30.5	24.0
12 244	A (10) + 747 (0.6)	41.3	34.8
13 247	V	56.1	49.6
14 262	A (10)	48.6	42.1
15 256	747 (0.2)	52.2	45.7
16 267	747 (0.6)	46.3	39.8
17 268	A (10) + 747 (0.3)	42.2	35.7
18 242	A (10) + 747 (0.6)	21.6	15.1

V = Vehicle

A = Aspirin

747 = CS747

#1
252.9g
251.7g
262.3g
267.3g
256.4g
271.1g

八八〇ノン

#7
258.8g
245.2g
257.2g
269.0g
268.5g
244.4g

1000 u/ml soln. 1 ml + saline 9 ml = 100 units/ml

100 u/ml soln. 1 ml + saline 7 ml = 30 units/ml

#13
247.0g
262.3g
256.2g
267.3g
267.6g
242.1g

#5 0.0

#6 0.0

151

ラット AV shunt 血栓モデル
(アスピリル + CS747)

CS747

プロトコル p146, 147 試験 p110, 118

ケージ番号: 6703-01-01

性別、系統: ♂ SD
周年令: 7週

体重: 36 g

業者名: 日本 SLC

入荷日: [REDACTED]

実験者: 杉立 取扱

飼育期間: [REDACTED]

受付番号: 034163

匹数: 36

B.w.

72.0 mg
11.3 mg+1 271.0g
277.6g
272.8g
275.0g
245.5g
+6 251.6g

1224.1 mg

+10 248.2g
48 263.9g
49 253.3g
47 261.1g
411 263.4g
417 263.4g

5%アラビアゴム = 50 mg/ml

= 1224.1 / 24.48 ml dH₂O+12 271.3g
265.3g
266.0g
253.5g
245.6g
+13 272.4g

: Aspirin

10 mg/ml = 72.0 / 7.2 ml 5%アラビアゴム 50 mg/ml

ラット

SD ♂ (7wks 入荷)

日本 SLC

[REDACTED] 入荷

杉立

受付番号 034163

ラット

B.W. (g)

1 271

2 278

3 273

4 275

5 246

6 252

CO₂ gas で 安樂死

±±±

7 281

8 264

9 253

10 248

11 263

12 263

13 271

14 265

15 266

16 256

17 246

18 272

153

#1	0.0556 g	#7	0.0590 g	#13	0.0578 g
#2	0.0529 g	#8	0.0632 g	#14	44.3 mg
#3	0.0439 g	#9	0.0471 g	#15	0.0464 g
#4	0.0410 g	#10	0.0385 g	#16	42.2 mg
#5	0.0246 g	#11	0.0367 g	#17	48.3 mg
#6	0.0197 g	#12	0.0356 g	#18	36.6 mg

Thrombus weight (mg)

Aspirin + CS

veh

As

CS

As

As



A. Sugishita

Page 145

[REDACTED]

CS 747

+

Combination Experiment

Aspirin

Dose	CS 747	0.3 mg/kg (4hr)	about 40 mg
		1 mg/kg (4hr)	about 20

Dose in which both bleeding time and aggregation were measured:

Aspirin 10 + CS 747 0.6 2hr

First, experiment with a central focus on Aspirin 10 + CS 747 0.6

Another group 0.3 or 1

Conduct 0.3 because 1 seems to work too much.

Page 146

Arterio-venous Shunt Thrombosis Model in Rats
(Examination of effect by combination of CS-747 with aspirin)

[Object]

Examine the effect by combination of CS-747 with aspirin using Arterio-venous shunt thrombosis model in rats.

[Experimental Term]

Thirty six rats received on [REDACTED] are used.

[Animals]

Seven-week-old male SD rats (Japan SLC) are purchased and used for the experiment after preliminary breeding for about a week. The experiment is conducted as 6 rats per group.

[Test agents]

CS-747 (synthesized by Ube Industries Ltd., Lot No. 16) and aspirin (Sigma, A-5376, Lot No. 46H1053, received on [REDACTED]) are used. The test agents are dissolved or suspended in a 5% Arabic gum (Sigma, Lot No. 73H0705, opened on [REDACTED]) solution and administered orally in volume of 1 ml/kg two hours before starting arterio-venous shunt. Administered group are (A) vehicle, (B) aspirin 10 mg/kg, (C) CS-747 0.3 mg/kg, (D) CS-747 0.6 mg/kg, (E) aspirin 10 mg/kg + CS-747 0.3 mg/kg, and (F) aspirin 10 mg/kg + CS-747 0.6 mg/kg.

[Methods]

- (1) For the experiment, the method by Umetsu et al. (Thromb. Haemost. 39, 74-83, 1978) is partly modified.
- (2) The shunt tube for arterio-venous shunt is prepared as follows: both sides of a medical silicon tube of 12 cm length (inner diameter: 1.5 mm, outer diameter: 2.5 mm, KANEKA Medix Co., Ltd) are connected each to a polyethylene tube of 7 cm length (inner diameter: 0.5 mm, outer diameter: 1.0 mm, Natsume Seisakusho Co., Ltd.)

covered with silicon via a medical silicon tube of 0.7 cm length (inner diameter: 1.0 mm, outer diameter: 1.5 mm, KANEKA Medix Co., Ltd.) as connector. At the connection,

Page 147

[REDACTED]

surgical adhesive (Aronalpfa A, Sankyo) is used for preventing blood leak. In addition, a silk thread (size 3-0, Niccho Kogyo) of 10 cm length is placed in the tube of 12 cm length.

(3) Vehicle (5% Arabic gum solution) or test agents are administered orally in a volume of 1 ml/kg 2 hours before starting arterio-venous shunt. 6 rats per group are used.

(4) The above tube prepared in advance is filled with heparin solution (Japanese Pharmacopoeia Heparin Sodium Injection, Fuso Pharmaceutical Industries, Ltd., Lot No. 97H28A, received on [REDACTED]) diluted with normal saline (Otsuka) resulting in 30 unit/kg.

(5) The rat is anesthetized with an intraperitoneal injection of 1 ml/kg (40 mg/kg) of pentobarbital solution (Nembutal R, Abbott, Lot No. 20-975-Z7) diluted with normal saline resulting in 40 mg/ml. After it is fixed to turning up, the jugular vein is exposed and one side of the shunt tube (in which the silk thread is not adhered) is cannulated. Subsequently, to the carotid artery where bloodstream is shut using clamp, the other side of the tube is cannulated to make the arterio-venous shunt.

(6) After removing the clamp and allowing blood to circulate for 30 minutes, the thrombus adsorbed on the silk thread is weighed. The thrombus weight was calculated by subtracting of the weight of the thread (6.5 mg) from the measured weight.

Files were stored at AV-shunt (3) (F00515 data)

[REDACTED] A. Sugidachi

Page 148

AV shunt Thrombosis Model in Rats
(Combination of CS 747 with aspirin)

Protocol: P. 146, 147

Reagent and so on: p. 110, p. 138

Cage number: 6raC3-01-04

Rats

SD male (Japan SLC)

Sex, system: male SD

[REDACTED] wks, Receipt

Year-round old: 7 weeks

Receipt number: 034163

Body weight:

Sugidachi

Manufacture name: Japan SLC

Receive date: [REDACTED] Number of rats: 36

Experimenter: Atsuhiko Sugidachi

Housing term: [REDACTED] to [REDACTED]

Receipt number: 034163

5% Arabic gum soln.

57.7 mg aspirin 50 mg/ml = 1011.7 mg / 20.234 ml dH₂O

16.2 mg CS 747

Aspirin

10 mg/ml = 57.7 mg / 5.77 ml 5% Arabic gum soln.

1011.7 mg Arabic gum

CS 747

16.2 mg / 5.4 ml = 3 mg/ml

3 mg/ml soln. 1 ml + 5% Arabic gum soln. 2 ml = 1 mg/ml

1 mg/ml soln. 1 ml + 5% Arabic gum soln. 2.33 ml = 0.3 mg/ml

1 mg/ml soln. 1.5 ml + 5% Arabic gum soln. 1 ml = 0.6 mg/ml

10.7 mg CS 747

Further prepared because of insufficient (spilled)

5% Arabic gum 1084.7 mg / 21.69 ml dH₂O = 50 mg/ml

1084.7 mg Arabic gum

CS 747 10.7 mg / 10.7 ml Arabic gum soln. = 1 mg/ml

1 mg/ml soln. 1.5 ml + 5% Arabic gum soln. 3.5 ml = 0.3 mg/ml

1 mg/ml soln. 3 ml + 5% Arabic gum soln. 2 ml = 0.6 mg/ml

Page 149

	B. W. (g)	Treatment	Measured value (mg)	Thrombus (mg)
#1	253	Vehicle	61.3	54.8
2	252	Aspirin 10	51.3	44.8
3	262	CS 747 0.3	58.3	51.8
4	267	CS 747 0.6	43.1	36.6
5	256	Aspirin 10 + CS 747 0.3	39.6	33.1
6	271	Aspirin 10 + CS 747 0.6	23.2	16.7
7	250	V	63.2	56.7
8	246	A 10	58.4	51.9
9	258	747 0.3	51.8	45.3
10	269	747 0.6	53.1	46.6
11	268	A 10 + 747 0.3	30.5	24.0
12	244	A 10 + 747 0.6	41.3	34.8
13	247	V	56.1	49.6
14	262	A 10	48.6	42.1
15	256	747 0.3	52.2	45.7
16	267	747 0.6	46.3	39.8
17	268	A 10 + 747 0.3	42.2	35.7
18	242	A 10 + 747 0.6	21.6	15.1

V = Vehicle

A = Aspirin

747 = CS 747

heparin

1000 u/ml soln. 1 ml + saline 9 ml = 100 unit/ml

100 u/ml soln. 3 ml + saline 7 ml = 30 unit/ml

#1 252.9 g
 251.7 g
 262.3 g
 267.3 g
 256.4 g
 271.1 g

#7 250.0 g
 245.8 g
 257.8 g
 269.0 g
 268.3 g
 244.4 g

#13 247.0 g
 262.3 g
 256.2 g
 267.3 g
 267.6 g
 242.1 g

Page 150

[REDACTED]
#1 0.0613 g #7 0.0632 g #13 0.0561 g

#2 0.0513 g #8 0.0584 g #14 48.6 mg

#3 0.0583 g #9 0.0518 g #15 52.2 mg

#4 0.0431 g #10 0.0531 g #16 0.0463 g

#5 0.0396 g #11 0.0305 g #17 0.0422 g

#6 0.0232 g #12 41.3 mg #18 0.0216 g

[REDACTED] A. Sugidachi

Page 151

AV Shunt Thrombosis Model in Rats
 (Aspirin + CS 747)

Protocol: p. 146, 147

Reagents and so on: p. 110, 138

Cage number:	6raC3-01-01	Rat
		6 SD male (7 wks, Receipt)
Sex, system:	male SD	36+2 Japan SLC
Year-round old:	7 weeks	Receipt
Body weight:		Sugidachi
Manufacture name:	Japan SLC	Receipt number: 034163
Receive date:		Number of rats: 36
Experimenter:	Atsuhiko Sugidachi	
Housing term:	to	
Receipt number:	034163	

Additional 2 rats

↓

euthanasia using CO₂ gas

	B. W.	
#1	271.0 g	72.0 mg
	277.6 g	11.3 mg
	272.8 g	
	275.0 g	
	245.5 g	
#6	251.6 g	1224.1 mg
#10	248.2 g	
#8	263.9 g	
#9	253.3 g	
#7	281.1 g	
#11	263.4 g	5% Arabic gum = 50 mg/ml
#12	263.4 g	= 1224.1 / 24.48 ml dH ₂ O
#13	271.3 g	
	265.3 g	
	266.0 g	
	255.5 g	Aspirin
	245.6 g	10 mg/ml = 72.0 / 7.2 ml 5% Arabic gum soln.
#18	272.4 g	

Page 152

CS 747 11.3 mg / 11.3 ml 5% Arabic gum soln. = 1 mg/ml
 1 mg/ml soln. 1.5 ml + 5% Arabic gum soln. 1ml = 0.6 mg/ml
 1 mg/ml soln. 1 ml + 5% Arabic gum soln. 2.33 ml = 0.3 mg/ml

heparin 1000 unit/ml soln. (origine) 1 ml + saline 9 ml = 100 unit/ml
 100 unit/ml soln. 3 ml + saline 7 ml = 30 unit/ml

	B. W. (g)	Treatment	Measured value (mg)	Thrombus (mg)
#1	271	V	55.6	49.1
2	278	A [10]	52.9	46.4
3	273	747 [0.3]	43.9	37.4
4	275	747 [0.6]	41.0	34.5
5	246	A [10] + 747 [0.3]	24.6	18.1
6	252	A [10] + 747 [0.6]	19.7	13.2
7	281	V	59.0	52.5
8	264	A [10]	63.2	56.7
9	253	747 [0.3]	47.1	40.6
10	248	747 [0.6]	38.5	32.0
11	263	A [10] + 747 [0.3]	36.7	30.2
12	263	A [10] + 747 [0.6]	35.6	29.1
13	271	V	57.8	51.3
14	265	A [10]	44.3	37.8
15	266	747 [0.3]	46.4	39.9
16	256	747 [0.6]	42.2	35.7
17	246	A [10] + 747 [0.3]	48.3	41.8
18	272	A [10] + 747 [0.6]	36.6	30.1

V = Vehicle

A = Aspirin

747 = CS 747

Page 153

#1 0.0556 g #7 0.0590 g #13 0.0578 g

#2 0.0629 g #8 0.0632 g #14 44.3 mg

#3 0.0439 g #9 0.0471 g #15 0.0464 g

#4 0.0410 g #10 0.0385 g #16 42.2 mg

#5 0.0246 g #11 0.0367 g #17 48.3 mg

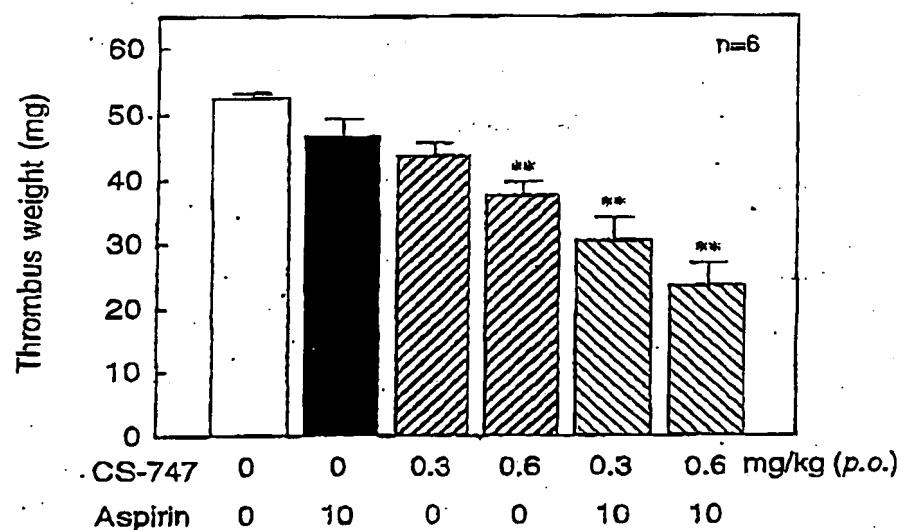
#6 0.0197 g #12 0.0356 g #18 36.6 mg

A. Sugidachi

Hirose

Page 154

Arterio-venous shunt thrombosis model in rats



Aspirin + CS 747 Summary

Vehicle	52.3 ± 1.2
Aspirin [10]	46.6 ± 2.8
CS 747 [0.3]	43.5 ± 2.1
CS 747 [0.6]	37.5 ± 2.1
Aspirin [10] + CS 747 [0.3]	30.5 ± 3.5
Aspirin [10] + CS 747 [0.6]	23.2 ± 3.8

A. Sugidachi